

**WHAT IS CLAIMED IS:**

- ✓ 1. An ORF selection vector comprising:
- 5 (a) a promoter;
- (b) a start codon operably linked to the promoter;
- (c) a reporter gene that is positioned downstream from both the promoter and the start codon and is out of frame.
- 10 2. The ORF selection vector of claim 1, wherein a nucleic acid sequence is inserted between the start codon and the reporter gene such that the reporter gene is in frame.
3. The ORF selection vector of claim 2, wherein the inserted nucleic acid sequence is genomic DNA.
- 15 4. The ORF selection vector of claim 3, wherein the genomic DNA is from a eukaryote.
5. The ORF selection vector of claim 3, wherein the genomic DNA is from a prokaryote.
- 20 6. The ORF selection vector of claim 2, wherein the genomic DNA is from a pathogen.

7. The ORF selection vector of claim 6, wherein the genomic DNA is from a parasite.

8. The ORF selection vector of claim 7, wherein the parasite is *Plasmodium falciparum*.

9. The ORF selection vector of claim 7, wherein the parasite is *Neospora caninum*.

10. The ORF selection vector of claim 7, wherein the parasite is *Trypanosoma cruzi*.

11. The ORF selection vector of claim 1, wherein the reporter gene lacks a start codon.

12. The ORF selection vector of claim 1, wherein the reporter gene encodes a gene product that is nonenzymatic.

13. The ORF selection vector of claim 12, wherein the gene product is GFP.

14. The ORF selection vector of claim 1, wherein the reporter gene is a death gene.

15. The ORF selection vector of claim 14, wherein the death gene encodes an enzyme, a DNA replication inhibitor, or a membrane disruptor.

16. The ORF selection vector of claim 15, wherein the enzyme is barnase, colicin, or SacB.

5 17. The ORF selection vector of claim 15, wherein the DNA replication inhibitor is CcdB, Kid, or GATA.

18. The ORF selection vector of claim 15, wherein the membrane disruptor is Hok, holins, or granulysin.

10 19. The ORF selection vector of claim 14, wherein the death gene encodes Doc.

20. The ORF selection vector of claim 2, wherein the nucleic acid sequence is part or all of an ORF of a gene.

15 21. The ORF selection vector of claim 1, wherein the promoter is a T7 promoter.

22. The ORF selection vector of claim 1, further comprising a restriction endonuclease site between the start codon and the reporter gene.

20 23. The ORF selection vector of claim 1, further comprising an origin of replication.

24. The ORF selection vector of claim 1, further comprising a selectable marker.

25. The ORF selection vector of claim 24, wherein the selectable marker is in frame and expressed in a host cell.

- ✓ 26. A method of producing an ORF selection vector comprising:
- 5 (a) contacting genomic DNA with a restriction endonuclease;
- (b) obtaining an ORF selection vector comprising:
- (i) a promoter;
- (ii) a start codon, wherein the start codon operably linked to the promoter;
- 10 (iii) a reporter gene that is positioned downstream from both the promoter and the start codon and is out of frame;
- (c) contacting the ORF selection vector with a restriction endonuclease; and
- (d) ligating a genomic restriction endonuclease DNA fragment generated from step (a) with the linearized ORF selection vector.

15 27. The method of claim 26, further comprising transfecting a host cell with the ligated ORF selection vector.

28. The method of claim 27, wherein the host cell is a bacterial host cell.

20 29. The method of claim 26, wherein the ligated ORF selection vector is capable of expressing the reporter gene.

30. The method of claim 26, wherein the genomic restriction endonuclease DNA fragment comprises a portion of an ORF.

31. The method of claim 30, wherein the DNA fragment is from a eukaryote.

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32. The method of claim 30, wherein the DNA fragment is from a prokaryote.

33. The method of claim 30, wherein the DNA fragment is from a pathogen.

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34. The method of claim 30, wherein the DNA fragment is from a parasite.

35. The method of claim 34, wherein the parasite is *Plasmodium falciparum*.

36. The method of claim 34, wherein the parasite is *Neospora caninum*.

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37. The method of claim 34, wherein the parasite is *Trypanosoma cruzi*.

38. The method of claim 26, wherein the reporter gene lacks a start codon.

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39. The method of claim 26, wherein the reporter gene encodes a gene product that is nonenzymatic.

40. The method of claim 39, wherein the gene product is GFP.

41. The method of claim 26, wherein the reporter gene is a death gene.

42. The method of claim 41, wherein the death gene encodes an enzyme, a DNA  
5 replication inhibitor, or a membrane disruptor.

43. The method of claim 42, wherein the enzyme is barnase, colicin, or SacB.

44. The method of claim 42, wherein the DNA replication inhibitor is CcdB, Kid, or  
10 GATA.

45. The method of claim 42, wherein the membrane disruptor is Hok, holins, or  
granulysin.

46. The method of claim 41, wherein the death gene encodes Doc.  
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47. The method of claim 26, wherein the promoter of the ORF selection vector is a T7  
promoter.

48. The method of claim 26, wherein the restriction endonuclease contacted with the  
20 genomic DNA creates a site compatible with the site created by the restriction  
endonuclease contacted with the ORF selection vector.

49. The method of claim 26, further comprising contacting the ORF selection vector with a phosphatase after it is contacted with a restriction endonuclease.

✓ 50. A method of identifying at least a portion of an ORF comprising:

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- (a) contacting genomic DNA with a restriction endonuclease;
- (b) obtaining an ORF selection vector comprising:
  - (i) a promoter;
  - (ii) a start codon operably linked to the promoter;
  - (iii) a reporter gene that is positioned downstream from both the promoter and the start codon and is out of frame;
- (c) contacting the ORF selection vector with a restriction endonuclease;
- (d) ligating a genomic restriction endonuclease DNA fragment generated from step (a) with the linearized ORF selection vector;
- (e) transfecting a host cell with the ligated selection vector;
- (f) determining whether the reporter gene is expressed.

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51. The method of claim 50, wherein the genomic restriction endonuclease DNA fragment comprises a portion of an ORF.

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52. The method of claim 51, wherein the DNA fragment is from a eukaryote.

53. The method of claim 51, wherein the DNA fragment is from a prokaryote.

54. The method of claim 51, wherein the DNA fragment is from a pathogen.

55. The method of claim 51, wherein the DNA fragment is from a parasite.

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56. The ORF selection vector of claim 55, wherein the parasite is *Plasmodium falciparum*.

57. The ORF selection vector of claim 55, wherein the parasite is *Neospora caninum*.

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58. The ORF selection vector of claim 55, wherein the parasite is *Trypanosoma cruzi*.

59. The method of claim 50, wherein the reporter gene lacks a start codon.

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60. The method of claim 50, wherein the reporter gene is nonselectable.

61. The method of claim 60, wherein the reporter gene encodes a gene product that is nonenzymatic.

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62. The method of claim 61, wherein the gene product is GFP.

63. The method of claim 50, wherein the reporter gene is a death gene.



64. The method of claim 63, wherein the death gene encodes an enzyme, a DNA replication inhibitor, or a membrane disruptor.

65. The method of claim 64, wherein the enzyme is barnase, colicin, or SacB.

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66. The method of claim 64, wherein the DNA replication inhibitor is CcdB, Kid, or GATA.

67. The method of claim 64, wherein the membrane disruptor is Hok, holins, or granulysin.

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68. The method of claim 63, wherein the death gene encodes Doc.

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69. The method of claim 50, wherein the promoter of the ORF selection vector is a T7 promoter.

✓ 70. A method of inducing an immune response in an animal comprising:

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(a) obtaining an ORF selection vector comprising:

(i) a promoter;

(ii) a start codon operably linked to the promoter;

(iii) a reporter gene that is positioned downstream from both the promoter and the start codon;

(iv) at least a part of a genomic ORF that is positioned between the start codon and the reporter gene;

- 5 (b) identifying an ORF by determining whether the reporter gene is expressed;
- (c) if the reporter gene is expressed, subcloning the ORF into an expression construct lacking the reporter gene;
- (d) introducing the expression construct into an the animal in a manner effective to induce an immune response against one or more antigens that may be encoded by the construct.
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71. The method of claim 70, wherein the ORF is from a eukaryote.

72. The method of claim 71, where in the ORF is from a tumor cell.

73. The method of claim 70, wherein the ORF is from a prokaryote.

74. The method of claim 70, wherein the DNA fragment is from a pathogen.

20 75. The method of claim 70, wherein the DNA fragment is from a parasite.

76. The method of claim 75, wherein the parasite is *Plasmodium falciparum*.

77. The method of claim 75, wherein the parasite is *Neospora caninum*.

78. The method of claim 75, wherein the parasite is *Trypanosoma cruzi*.

5 79. The method of claim 70, wherein the reporter gene lacks a start codon.

80. The method of claim 70, wherein the reporter gene encodes a gene product that is nonenzymatic.

10 81. The method of claim 80, wherein the gene product is GFP.

82. The method of claim 70, wherein the reporter gene is toxic to a host cell.

15 83. The method of claim 70, wherein the promoter of the ORF selection vector is a T7 promoter.

84. The method of claim 70, wherein the expression construct contains a eukaryotic promoter.

20 85. The method of claim 84, wherein the eukaryotic promoter is from the same species as the animal.

86. The method of claim 70, further comprising testing the animal for an immune response.

87. The method of claim 86, wherein the testing comprises challenging the animal with an expression product of the ORF.

88. The method of claim 70, further comprising obtaining antibodies generated in response to one or more antigens encoded by the introduced second construct.

10 89. A method of preparing an antigen comprising:

(a) obtaining an ORF selection vector comprising:

(i) a promoter;

(ii) a start codon operably linked to the promoter;

(iii) a reporter gene that is positioned downstream from both the promoter and the start codon;

(iv) at least a part of a genomic ORF that is positioned between the start codon and the reporter gene;

(b) identifying an ORF by determining whether the reporter gene is expressed;

20 (c) if the reporter gene is expressed, subcloning the ORF into an expression construct lacking the reporter gene;

(d) administering to an animal a pharmaceutical composition comprising one or more expression constructs; and

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[illegible]

(i)

(ii)

(iii)

(ix)

(b)